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Attorney Docket No. 87714/113

In re patent application of

Thyagarajan Srikantha *et al.*

Serial No.: 09/424,951

Group Art Unit: 1636

Filed: January 20, 2000

Examiner: Katharine Davis

For: Candida Albicans Two-Component Hybrid Kinase Gene, CaNIK1 and Use  
Thereof.

**DECLARATION UNDER 37 CFR § 1.131**

I, Thyagarajan Srikantha, declare that:

1. I am a citizen of the United States of America, residing at 1488 Valley View Drive, Coral Village, Iowa 522401.

2. I am employed at the Department of Biological Sciences, at the University of Iowa, Room 314, Iowa City Iowa, 52242

3. I am a co-inventor named in U.S. application Serial No. 09/424,951. In relation to the application, I have reviewed an Office Action, mailed May 24, 2001 and U.S. Patent No. 5,939,306 issued August 17, 1999 cited in that Office Action.

4. Exhibit A, entitled "PCR Amplification to Determine a CaNIK1 Probe," contains notebook entries, with the dates removed, that show the creation prior to April 16, 1997 of a *C. albicans* polynucleotide CaNIK1 "two component histidine kinase homologue" probe, using primers that have homology to two highly conserved regions of "two component response regulator" proteins in bacteria. These same data appear in Example I of the present application.

Serial No. 09/228,958

5. Exhibit B, entitled "Isolation of the CaNIK1 Gene," contains notebook entries that document (1) the identification of the CaNIK1 gene in *E. coli* strain containing the lambda phage  $\phi$ SA15.1, using the probe obtained from the experiments discussed in Exhibit A. These same data appear in Example 2 of the current application.

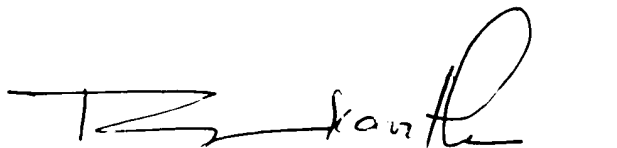
6. Exhibit C, entitled "Determination of the full-length sequence of the CaNIK1 Gene," contains notebook entries that confirm the acquisition of the nucleotide sequence of the isolated CaNIK1 gene of exhibit B. These same data are shown in Example 2 and Figure 2 A-C of the present application.

7. All experiments described in Exhibits A-C were conducted under my direction and supervision or that of my coinventor, Dr. David Soll.

8. This project was diligently carried out without delay from prior to April 16, 1997 until its completion.

9. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

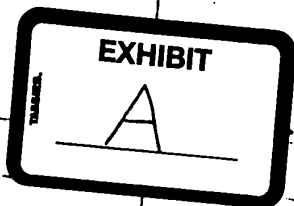
July 23<sup>rd</sup> 2001  
Dated

  
Thyagarajan Srikantha



## Exhibit A

PCR Amplification to Determine a CaNIK1 Probe



# Isolation of McMI/SLNI homologues from C. alb and C. glabrata

## McMI oligo's:

Conserved region between S. cere and S. pombe  
McMI and NAPI genes containing  
NADS box: Mc5' and Mc3'

## SLNI oligo's:

Designed based on ① Histidine auto phosphorylation domain (SRB1) and ② ATP-binding motif (SRB2) and ③ Aspartyl-phosphate acceptor domain motif (SRB3)

S = SLNI, ~~SLNI~~ Lema, B, BarA homologues were considered.  
PCR reactions.



94/40/72  
15/2/3'  
40 cycles

only  
SLNI PCR  
products seen

# Use of Ultima for SLN PCR.

Template	1x		
10x buf	10 $\mu$ l	40	1 $\rightarrow$ Sc 1+2
25mM MgCl <sub>2</sub>	10 $\mu$ l	40	2 $\rightarrow$ CA 1+2
10mM dNTP	8 $\mu$ l	32	3 $\rightarrow$ CG 1+2
Sm P <sub>1</sub>	1 $\mu$ l	4	4 $\rightarrow$ Sc 1+3
Sm P <sub>2</sub>	2 $\mu$ l	8	5 $\rightarrow$ CA 1+2
Ultima	2 $\mu$ l	8	6 $\rightarrow$ CG 1+3
H <sub>2</sub> O	0.5 $\mu$ l	2	
	66.5 $\mu$ l	270 $\mu$ l	

## MiniPCR diag. PCR.

	1x	
10x $\rightarrow$	5	14
MgCl <sub>2</sub> $\rightarrow$	4	70
dNTP $\rightarrow$	0.5	56
P <sub>1</sub> $\rightarrow$	1	7
P <sub>2</sub>	1	14
Ult	0.1	14
H <sub>2</sub> O	1.5	1.5
	38.4	540 $\mu$ l

94 / 45 / 72°C  
1' 1' 2.5'  
45 cycles

## using GEN system

Repeat

94 / 40 / 72°C  
1.5' / 2' / 3'

40 cycles

# ligation of SLN-PCR product with T-easy vector:

	<u>SS<sub>1,2</sub></u>	<u>SS<sub>1,3</sub></u>	<u>SA<sub>1,2</sub></u>	<u>SA<sub>1,3</sub></u>	<u>SG<sub>1,2/1,3</sub></u>
T-Vector	1 $\mu$ l				
Insert	2.5 $\mu$ l				
10x buf	1.0 $\mu$ l				
ligase	1.0 $\mu$ l				
H <sub>2</sub> O	4.5 $\mu$ l				

5  $\mu$ l transformed into STBL2

X-gal plates  
good # of transformants.

## Diagnostic PCR with SP6/T7 Prime 125 $\mu$ l system.

DNA $\rightarrow$ 2.5 $\mu$ l	105	1' 15' 3'
10x buf $\rightarrow$ 2.5 $\mu$ l	84 $\mu$ l	$\rightarrow$ 94/45/72°C
25mM MgCl <sub>2</sub> $\rightarrow$ 2.0 $\mu$ l	10.5 $\mu$ l	40 cycles
10mM NTP $\rightarrow$ 0.25 $\mu$ l	21 $\mu$ l	
5 $\mu$ M SP6 $\rightarrow$ 0.5 $\mu$ l	21 $\mu$ l	
5 $\mu$ M T7 $\rightarrow$ 0.5 $\mu$ l	5.0 $\mu$ l	
Taq $\rightarrow$ 0.1 $\mu$ l	715 $\mu$ l	
H <sub>2</sub> O $\rightarrow$ 17.0 $\mu$ l		

# ds sequencing of plasmids.

		Template	Primer	
1. PSA1.2/5	→ Forward	2 $\mu$ l	—	5
	2 → Reverse	2 $\mu$ l	—	↓
1.2/16	3 → F	2 $\mu$ l	—	
	4 → R	2 $\mu$ l	—	
2. PCG1.3	5 → REN	3 $\mu$ l	0.5 $\mu$ l	7.5
	6 → G12	3 $\mu$ l	2.0 $\mu$ l	6.0
3. PCG2.1	7 → REN1	3 $\mu$ l	0.5 $\mu$ l	7.5
	8 → G12	3 $\mu$ l	2.0 $\mu$ l	6.0

10 20 30 40 50 60  
TNNNGNAATT GTAATACGAC TCACTATAGG GCGAATTGG CCGGACGTCG CATGCCCCG  
70 80 90 100 110 120  
GCCGCCATGG CGGCCGCGGG AATTCEATTG AATTGAGAAC GCCGGTGAAT GGGATTATTG  
130 140 150 160 170 180  
GAATGACCCA GTTGTCACTT GATACAGAGT TGACACAGTA CCAACGAGAG ATGTTGTCCA  
190 200 210 220 230 240  
TTGTGCATAA CTTGGCAAAT TCCTTGTTGA CCATTATAGA CGATATATTG GATATTTCTA  
250 260 270 280 290 300  
AGATTGAGGC GAATAGAATG ACGGTGGAAC AGATTGATTT TTCATTAAGA GGGACAGTGT  
310 320 330 340 350 360  
TTGGTGCAAT GAAAACGTTA GCCGTCAAAG CTATTGAAAA AAACCTAGAC TTGACCTATC  
370 380 390 400 410 420  
AATGTGATTC ATCGTTTCCA GATAATCTTA TTGGAGATAG TTTTAGATTA CGACAAGTTA  
430 440 450 460 470 480  
TTCTTAACTT GGCTGGTAAT GCTATTAAAGT TTAATAAAGA GGGGAAAGTT AGTGTTAGTG  
490 500 510 520 530 540  
TGAAAAAGTC TGATAAAATG GTGTTAGATA GTAAGTTGTT GTTAGAGGTT TGTGTTAGCG  
550 560 570 580 590 600  
ACACGGGAAT AGGTATAGAG AAAGACAAAT TGGGATTGAT TTTCGATACC TTCTGTCAAG  
610 620 630 640 650 660  
CTGATGGTTC TACTACAAGA AAGTTTGGTG GTACTGGCTT ANGCTAATC ACTAGTGAAT  
670 680 690 700 710 720  
TCGCGGCCGC CTGCAGGTCG ACCATATGGG ANAGCCCCAA CGCGTTGGAT GCATAGCTTG  
730 740 750 760 770 780  
AGTATCTATA GTGTCACTAA ATAGCTTG.....

Handwritten notes and markings:  
- *SL1* (arrow from 100 to 170)  
- *SL2* (arrow from 620 to 640)  
- *SL3* (arrow from 520 to 590)  
- *SP1* (arrow from 650 to 660)  
- *SP2* (arrow from 130 to 140)  
- *SP3* (arrow from 140 to 150)  
- *SP4* (arrow from 150 to 160)  
- *SP5* (arrow from 160 to 170)  
- *SP6* (arrow from 170 to 180)  
- *SP7* (arrow from 180 to 190)  
- *SP8* (arrow from 190 to 200)  
- *SP9* (arrow from 200 to 210)  
- *SP10* (arrow from 210 to 220)  
- *SP11* (arrow from 220 to 230)  
- *SP12* (arrow from 230 to 240)  
- *SP13* (arrow from 240 to 250)  
- *SP14* (arrow from 250 to 260)  
- *SP15* (arrow from 260 to 270)  
- *SP16* (arrow from 270 to 280)  
- *SP17* (arrow from 280 to 290)  
- *SP18* (arrow from 290 to 300)  
- *SP19* (arrow from 300 to 310)  
- *SP20* (arrow from 310 to 320)  
- *SP21* (arrow from 320 to 330)  
- *SP22* (arrow from 330 to 340)  
- *SP23* (arrow from 340 to 350)  
- *SP24* (arrow from 350 to 360)  
- *SP25* (arrow from 360 to 370)  
- *SP26* (arrow from 370 to 380)  
- *SP27* (arrow from 380 to 390)  
- *SP28* (arrow from 390 to 400)  
- *SP29* (arrow from 400 to 410)  
- *SP30* (arrow from 410 to 420)  
- *SP31* (arrow from 420 to 430)  
- *SP32* (arrow from 430 to 440)  
- *SP33* (arrow from 440 to 450)  
- *SP34* (arrow from 450 to 460)  
- *SP35* (arrow from 460 to 470)  
- *SP36* (arrow from 470 to 480)  
- *SP37* (arrow from 480 to 490)  
- *SP38* (arrow from 490 to 500)  
- *SP39* (arrow from 500 to 510)  
- *SP40* (arrow from 510 to 520)  
- *SP41* (arrow from 520 to 530)  
- *SP42* (arrow from 530 to 540)  
- *SP43* (arrow from 540 to 550)  
- *SP44* (arrow from 550 to 560)  
- *SP45* (arrow from 560 to 570)  
- *SP46* (arrow from 570 to 580)  
- *SP47* (arrow from 580 to 590)  
- *SP48* (arrow from 590 to 600)  
- *SP49* (arrow from 600 to 610)  
- *SP50* (arrow from 610 to 620)  
- *SP51* (arrow from 620 to 630)  
- *SP52* (arrow from 630 to 640)  
- *SP53* (arrow from 640 to 650)  
- *SP54* (arrow from 650 to 660)  
- *SP55* (arrow from 660 to 670)  
- *SP56* (arrow from 670 to 680)  
- *SP57* (arrow from 680 to 690)  
- *SP58* (arrow from 690 to 700)  
- *SP59* (arrow from 700 to 710)  
- *SP60* (arrow from 710 to 720)  
- *SP61* (arrow from 720 to 730)  
- *SP62* (arrow from 730 to 740)  
- *SP63* (arrow from 740 to 750)  
- *SP64* (arrow from 750 to 760)  
- *SP65* (arrow from 760 to 770)  
- *SP66* (arrow from 770 to 780)

558bp



10	20	30	40	50	60
ATGGATGACG	CCAAACTATT	TAGGTGACAC	TATAGAATAC	TCAAGCTATG	CATCCAACGC
70	80	90	100	110	120
GTTGGGAGCT	CTCCCATATG	GTCGACCTGC	AGGCGGCCGC	GAATTCACCTA	GTGATTAGGC
130	140	150	160	170	180
CTAAGCCAGT	ACCACCAAAC	TTTCTTGTAG	TAGAACCATC	AGCTTGACAG	AAGGTATCGA
190	200	210	220	230	240
AAATCAATCC	CAATTTGTCT	TTCTCTATAC	CTATTCCCGT	GTCGCTAACA	CAAACCTCTA
250	260	270	280	290	300
ACAACAACIT	ACTATCTAAC	ACCATTTTAT	CAGACTTTTT	CACACTAACA	CTAACTTTCC
310	320	330	340	350	360
CCTCTTTAGT	AAACTTAATA	GCATTACCAG	CCAAGTTAAG	AATAACTTGT	CGTAATCTAA
370	380	390	400	410	420
AACTATCTCC	AATAAGATTA	TCTGGAAACG	ATGAATCACA	TTGATAGGTC	AAGTCTAGGT
430	440	450	460	470	480
TTTTTTCAAT	AGCTTTGACG	GCTAACGTTT	TCAATGCACC	AAACACTGTC	CCTCTTAATG
490	500	510	520	530	540
AAAAATCAAT	CTGTTCCACC	GTCATTCTAT	TCGCCTCAAT	CTTAGAAATA	TCCAATATAT
550	560	570	580	590	600
CGTCTATAAT	GGTCAACAAG	GAATTTGCCA	AGTTATGCAC	AATCGACAAC	ATCTCTCGTT
610	620	630	640	650	660
GGTACTGTGT	CAACTCTGTA	TCAAGTGACA	ACTGGGTCAT	TCCAATAATC	CCATTCCACCG
670	680	690	700	710	720
GCGTTCTCAA	TTCAATCGAA	TTCCCGCGGC	CGCATGGCGG	GCGGGAACAT	GCGACNTCGG
730	740	750	760	770	780
GCCAATTCGC	CCTATANTGA	GTCGTA....	.....	.....	.....

**Exhibit B**

**Isolation of the CaNIK1 Gene**

EXHIBIT

B

Screening of C. alb genomic library  
for ~~the~~ walking in 5' direction of  
the gene.

Dilute 8  $\mu$ l / 500  $\mu$ l SM.

Use 10  $\mu$ l  $\phi$  + 200  $\mu$ l cells.  
(P2)

5 plates

Probed with 1.384 probe

Diagnostic PCR  
Performed

25 +ve clones isolated

250  $\mu$ l SM ( $\sim$  15 plaques /  $\mu$ l)

Plated 1:2500 dilution

4  $\mu$ l  $\rightarrow$  10  $\mu$ l of  $\phi$  + 120  $\mu$ l P2

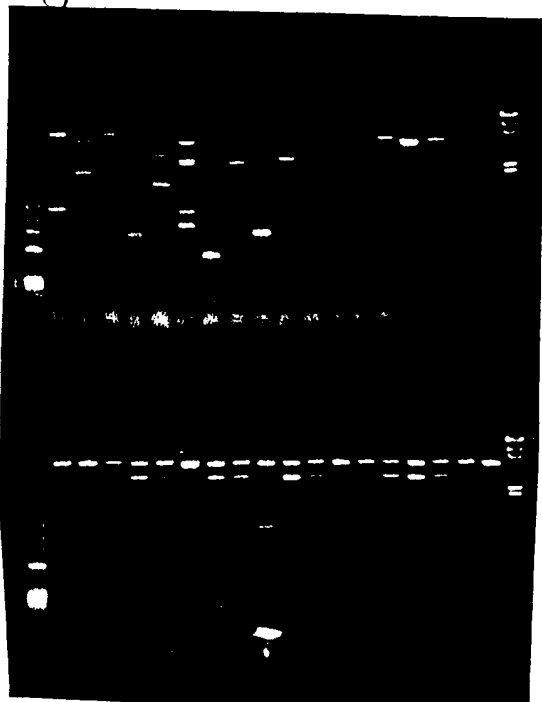
Probe  $\rightarrow$  1.384

Clones #2, #8, #10, #22, #18  
lysed  $\phi$ , #15, #1 showed  
+ve signals.

Single plaques were resuspended  
in 250  $\mu$ l SM.  
25  $\mu$ l of lysates + 150  $\mu$ l P2 were  
used to prepare plate lysates

1  
2  
3  
8  
10  
12  
15  
18

Diagnostic PCR 1 clone 1 time 18



Top Row  $\lambda_{SA1-18} \pm ELA + SLB4R$

bot Row  $\lambda_{SA1-18} \pm ERA + SLB4R$

Long PCR system  $\pm$

0.25  $\mu$ l Taq + 0.25  $\mu$ l long PCR enz

~~Q~~

Total 30 cycles.

## Exhibit C

### Determination of the Full-Length Sequence of the CaNIK1 Gene

5 9 18 27 36 45 54  
ATG GAG TTA ANT TTA AGG GNG AGG GCA GGA GTA TTG TTG GGT GAA ATA GGG GAG  
V E L X L R X R A G V L L G E I G E  
W S \* X \* G X G Q E Y C W V K \* G S  
G V X F K G E G R S I V G \* N R G V

63 72 81 90 99 108  
TGT GGT GGT TGA GAA GGG AGA GAG TTA TGA AAT TGG GGC ACA GGG TTT TTT TTT  
C G G \* E G R E L \* N W G T G F F F  
V V V E K G E S Y E I G A Q G F F F  
W W L R R E R V M K L G H R V F F F

117 126 135 144 153 162  
TTT TGG TTT TTC CTT TTT TAG TTG GGC ACC AAT ACT AAT TGA TTA TTT TTG CTA  
F W F F L F \* L G T N T N \* L F L L  
F G F S F F S W A P I L I D Y F C Y  
L V F P F L V G H Q Y \* L I I F A I

171 180 189 198 207 216  
TTA TGG GCA AAT TGG ATG TGG CAG AAT TTG CAA AAC AGA TCC GGA TAT TGT CAT  
L W A N W M W Q N L Q N R S G Y C H  
Y G Q I G C G R I C K T D P D I V M  
← M G K L D V A E F A K Q I R I L S W

225 234 243 252 261 270  
GG CAA GTC AGA AAT TAG TAG GTA TGG CAG TTG GAT GGT GTA GGG TAC TGG CAG  
G Q V R N \* \* V W Q L D G V G Y W Q  
← G K S E I S R Y G S W M V \* G T G R  
← A S Q K L V G M A V G W C R V L A G

279 288 297 306 315 324  
GCC CGG TAA AGG AAA CTA AAC ATG TTA GCC AGG GAG TGA GTC CTA CGG GGG GGA  
A R \* R K L N M L A R E \* V L R G G  
← P G K G N \* T C \* P G S E S Y G G E  
← P V K E T K H V S Q G V S P T G G S

333 342 351 360 369 378  
GCA TAG TAC ACC AGT AGT AGG ACC TCC TTT/GTT GGT CGT GTG TGG TGG CAA TAT  
A \* Y T S S R T S F V G R V W W Q Y  
H S T P V V G P P L L V V C G G N I  
I V H Q \* \* D L L C W S C V V A I Y

387 396 405 414 423 432  
ACA AAG CCC CCC CTT ACA TCA ACA AAA AAT TTT TCC TCT CTG TTG TTT ATC TCG  
T K P P L T S T K N F S S L L F I S  
→ Q S P P L H Q Q K I F P L C C L S R  
A → K A P P Y I N K K F F L S V V Y L E

441 450 459 468 477 486  
GT TTC TCC CTC TCT CTC CAA TGG CAC CCC ACT AAA AAA CCA CGG TTA TCA CCA  
S F S L S L Q W H P T K K P R L S P  
V S P S L S N G A T P L K N H G Y H Q  
→ F L P L S P M A P H \* K T T V I T N

SLB 16

SLB 17

495 504 513 522 531 540  
ATG CAG CCC TCT GTT TTT GAA ATA CTC AAC GAC CCT GAG CTT TAT AGT CAG CAC  
→ M Q P S V F E I L N D P E L Y S Q H  
C S P L F L K Y S T T L S F I V S T  
A A L C F \* N T Q R P \* A L \* S A L

549 558 567 576 585 594  
TGT CAT AGC CTT AGG GAA ACA CTT CTT GAT CAT TTC AAC CAT CAA GCT ACA CTT  
→ C H S L R E T L L D H F N H Q A T L  
V I A L G K H F L I I S T I K L H L  
S \* P \* G N T S \* S F Q P S S Y T Y

603 612 621 630 639 648  
ATC GAC ACT TAT GAA CAT GAA CTA GAA AAA TCC AAA AAT GCC AAC AAA GCG TCC  
→ I D T Y E H E L E K S K N A N K A S  
S T L M N M N \* K N P K M P T K R P  
R H L \* T \* T R K I Q K C Q Q S V P

657 666 675 684 693 702  
CAA CAA GCA CTT AGT GAA ATA GGT ACA GTT GTT ATA TCT GTT GCC ATG GGA GAC  
→ Q Q A L S E I G T V V I S V A M G D  
N K H L V K \* V Q L L Y L L P W E T  
T S T \* \* N R Y S C Y I C C H G R L

711 720 729 738 747 756  
TTG TCG AAA AAA GTT GAG ATT CAC ACA GTA GAA AAT GAC CCT GAG ATT TTA AAA  
→ L S K K V E I H T V E N D P E I L K  
C R K K L R F T Q \* K M T L R F \* K  
V E K S \* D S H S R K \* P \* D F K S

765 774 783 792 801 810  
GTC AAA ATC ACC ATC AAC ACC ATG ATG GAT CAA TTA CAG ACA TTT GCT AAT GAG  
→ V K I T I N T M M D Q L Q T F A N E  
S K S P S T P \* W I N Y R H L L M R  
Q N H H Q H H D G S I T D I C \* \* G

819 828 837 846 855 864  
GTT ACA AAA GTC GCC ACC GAA GTC GCA AAT GGT GAA CTA GGT GGA CAA GCG AAA  
→ V T K V A T E V A N G E L G G Q A K  
L Q K S P P K S Q M V N \* V D K R K  
Y K S R H R S R K W \* T R W T S E K

873 882 891 900 909 918  
AAT GAT GGA TCT GTT GGT ATT TGG AGA TCA CTT ACA GAC AAT GTT AAT ATT ATG  
→ N D G S V G I W R S L T D N V N I M  
M M D L L V F G D H L Q T M L I L W  
\* W I C W Y L E I T Y R Q C \* Y Y G

927 936 945 954 963 972  
GCT CTT AAT TTA ACT AAC CAA GTG CGA GAA ATT GCT GAT GTC ACA CGT GCT GTT  
→ A L N L T N Q V R E I A D V T R A V  
L L I \* L T K C E K L L M S H V L L  
S \* F N \* P S A R N C \* C H T C C C

```

      981      990      999      1008      1017      1026
GCC AAG GGG GAC TTG TCA CGT AAA ATT AAT GTA CAC GCC CAG GGT GAA ATC CTT
-----
A  K  G  D  L  S  R  K  I  N  V  H  A  Q  G  E  I  L
P  R  G  T  C  H  V  K  L  M  Y  T  P  R  V  K  S  F
Q  G  G  L  V  T  *  N  *  C  T  R  P  G  *  N  P  S

      1035      1044      1053      1062      1071      1080
CAA CTT CAA CGT ACA ATA AAC ACC ATG GTG GAT CAG TTA CGA ACG TTT GCA TTC
-----
Q  L  Q  R  T  I  N  T  M  V  D  Q  L  R  T  F  A  F
N  F  N  V  Q  *  T  P  W  W  I  S  Y  E  R  L  H  S
T  S  T  Y  N  K  H  H  G  G  S  V  T  N  V  C  I  R

      1089      1098      1107      1116      1125      1134
GAA GTA TCT AAA GTT GCT AGA GAT GTT GGT GTG CTT GGT ATA TTA GGA GGA CAA
-----
E  V  S  K  V  A  R  D  V  G  V  L  G  I  L  G  G  Q
K  Y  L  K  L  L  E  M  L  V  C  L  V  Y  *  E  D  K
S  I  *  S  C  *  R  C  W  C  A  W  Y  I  R  R  T  S

      1143      1152      1161      1170      1179      1188
GCG TTG ATT GAA AAT GTT GAA GGT ATT TGG GAA RAR TTG ANT GAT AAT GTC AAT
-----
A  L  I  E  N  V  E  G  I  W  E  X  L  X  D  N  V  N
R  *  L  K  M  L  K  V  F  G  K  X  *  X  I  M  S  M
V  D  *  K  C  *  R  Y  L  G  X  X  X  *  *  C  Q  C

      1197      1206 ACT      1215 GTG      1224      1233      1242 ACT
GCC ATG GCT CTT AAT TTG ANT ACA CAA NTG AGA AAT ATT GCC AAT GTC ACC NCT
-----
A  M  A  L  N  L  X  T  Q  X  R  N  I  A  N  V  T  X
P  W  L  L  I  *  X  H  X  *  E  I  L  P  M  S  P  L
H  G  S  *  F  X  Y  T  X  E  K  Y  C  Q  C  H  X  C

      1251      1260 ACT GTG      1269      1278 ACT GTG      1287 AAG      1296
GCC GTT GCC AAG GGG GAT TTG TCG AAA AAA GTC MCN NCT GAT TGT ANG GGA GAA
-----
A  V  A  K  G  D  L  S  K  K  V  X  X  D  C  X  G  E
P  L  P  R  G  I  C  R  K  K  S  X  L  I  V  X  E  K
R  C  Q  G  G  F  V  E  K  S  X  X  *  L  X  G  R  N

      1305      1314      1323      1332      1341      1350
ATY CTT GAT TTG AAA CTT ACT ATT AAT CAA ATG GTG GAC CGA TTA CAG AAT TTT
-----
I  L  D  L  K  L  T  I  N  Q  M  V  D  R  L  Q  N  F
X  L  I  *  N  L  L  L  I  K  W  W  T  D  Y  R  I  L
X  *  F  E  T  Y  Y  *  S  N  G  G  P  I  T  E  F  C

      1359      1368      1377      1386      1395      1404
GCT CTT GCG GTG ACG ACA TTG TCG AGA GAG GTT GGT ACT TTG GGT ATT TTG GGT
-----
A  L  A  V  T  T  L  S  R  E  V  G  T  L  G  I  L  G
L  L  R  *  R  H  C  R  E  R  L  V  L  W  V  F  W  V
S  C  G  D  D  I  V  E  R  G  W  Y  F  G  Y  F  G  W

      1413      1422      1431      1440      1449      1458
GGA CAA GCT AAC GTA CAG GAT GTT GAA GGT GCT TGG AAA CAG GTT ACA GAA AAT
-----
G  Q  A  N  V  Q  D  V  E  G  A  W  K  Q  V  T  E  N
D  K  L  T  Y  R  M  L  K  V  L  G  N  R  L  Q  K  M
T  S  *  R  T  G  C  *  R  C  L  E  T  G  Y  R  K  C

```



1467 1476 1485 1494 1503 1512  
GTC AAC CTA ATG GCT ACT AAT TTA ACT AAC CAA GTG AGA TCT ATT GCT ACA GTT  
-----  
V N L M A T N L T N Q V R S I A T V  
S T \* W L L I \* L T K \* D L L L Q L  
Q P N G Y \* F N \* P S E I Y C Y S Y

1521 1530 1539 1548 1557 1566  
ACT ACT GCA GTT GCG CAT GGT GAT TTG TCG CAA AAG ATT GAT GGT CAT CCC AAA  
-----  
T T A V A H G D L S Q K I D G H P K  
L L Q L R M V I C R K R L M V I P K  
Y C S C A W \* F V A K D \* W S S Q R

1575 1584 1593 1602 1611 1620  
GGA GAG ATT TTA CAA TTG AAA AAT ACA ATC AAC AAG ATG GTG GAC TCT TTG CAG  
-----  
G E I L Q L K N T I N K M V D S L Q  
E R F Y N \* K I Q S T R W W T L C S  
R D F T I E K Y N Q Q D G G L F A V

1629 1638 1647 1656 1665 1674  
TTG TTT GCA TCA GAA GTG TCG AAA GTG GCA CAA GAT GTT GGT ATT AAT GGA AAA  
-----  
L F A S E V S K V A Q D V G I N G K  
C L H Q K C R K W H K M L V L M E N  
V C I R S V E S G T R C W Y \* W K I

1683 1692 1701 1710 1719 1728  
TTA GGT ATT CAA GCA CAA GTT AGT GAT GTT GAT GGA TTA TCG AAG GAG ATT ACG  
-----  
L G I Q A Q V S D V D G L W K E I T  
\* V F K H K L V M L M D Y G R R L R  
R Y S S T S \* \* C \* W I M E G D Y V

1737 1746 1755 1764 1773 1782  
TCT AAT GTA AAT ACC ATG GCT TCA AAT TTA ACT TCG CAA GTG AGA GCT TTT GCA  
-----  
S N V N T M A S N L T S Q V R A F A  
L M \* I P W L Q I \* L R K \* E L L H  
\* C K Y H G F K F N F A S E S F C T

1791 1800 1809 1818 1827 1836  
CAG ATT ACT GCT GCT GCT ACT GAT GGG GAT TTC ACT AGA TTT ATT ACT GTT GAA  
-----  
Q I T A A A T D G D F T R F I T V E  
R L L L L L L M G I S L D L L L L K  
D Y C C C Y \* W G F H \* I Y Y C \* S

1845 1854 1863 1872 1881 1890  
GCA CTG GGA GAG ATG GAT GCG TTG AAA ACA AAG ATT AAT CAA ATG GTG TTT AAC  
-----  
A L G E M D A L K T K I N Q M V F N  
H W E R W M R \* K Q R L I K W C L T  
T G R D G C V E N K D \* S N G V \* L

1899 1908 1917 1926 1935 1944  
TTA AGG GAA TCG CTT CAA AGG AAT ACT GCG GCT AGA GAA GCT GCT GAG TTG GCC  
-----  
L R E S L Q R N T A A R E A A E L A  
\* G N R F K G I L R L E K L L S W P  
K G I A S K E Y C G \* R S C \* V G Q

1953 1962 1971 1980 1989 1998  
 AAT AGT GCG AAA TCC GAG TTT TTA GCA AAC ATG TCG CAT GAG ATT AGA ACA CCA  
 N S A K S E F L A N M S H E I R T P  
 I V R N P S F \* Q T C R M R L E H H  
 \* C E I R V F S K H V A \* D \* N T A I  
 2007 2016 2025 2034 2043 2052  
 TTG AAT GGG ATT ATT GGT ATG ACA CAG TTG TCR CTT GAT ACA GAG TTG ACR CMG  
 L N G I I G M T Q L S L D T E L T X  
 E W D Y W Y D T V V X \* Y R V D X V  
 2061 2070 2079 2088 2097 2106  
 TAC CAA CGA GAG ATG TTG TCG ATT GTG CAT AAC TTG GCA AAT TCC TTG TTG ACC  
 Y Q R E M L S I V H N L A N S L L T  
 T N E R C C R L C I T W Q I P C \* P  
 P T R D V V D C A \* L G K F L V D H  
 2115 2124 2133 2142 2151 2160  
 ATT ATA GAC GAT ATA TTG GAT ATT TCT AAG ATT GAG GCG AAT AGA ATG ACG GTG  
 I I D D I L D I S K I E A N R M T V  
 L \* T I Y W I F L R L R R I E \* R W  
 Y R R Y I G Y F \* D \* G E \* N D G G  
 2169 2178 2187 2196 2205 2214  
 GAA CAG ATT GAT TTT TCA TTA AGA GGG ACA GTG TTT GGT GCA TTG AAA ACG TTA  
 E Q I D F S L R G T V F G A L K T L  
 N R L I F H \* E G Q C L V H \* K R \*  
 T D \* F F I K R D S V W C I E N V S  
 2223 2232 2241 2250 2259 2268  
 GCC GTC AAA GCT ATT GAA AAA AAC CTA GAC TTG ACC TAT CAA TGT GAT TCA TCG  
 A V K A I E K N L D L T Y Q C D S S  
 P S K L L K K T \* T \* P I N V I H R  
 R Q S Y \* K K P R L D L S M \* F I V  
 2277 2286 2295 2304 2313 2322  
 TTT CCA GAT AAT CTT ATT GGA GAT AGT TTT AGA TTA CGA CAA GTT ATT CTT AAC  
 F P D N L I G D S F R L R Q V I L N  
 F Q I I L L E I V L D Y D K L F L T  
 S R \* S Y W R \* F \* I T T S Y S \* L  
 2331 2340 2349 2358 2367 2376  
 TTG GCT GGT AAT GCT ATT AAG TTT ACT AAA GAG GGG AAA GTT AGT GTT AGT GTG  
 L A G N A I K F T K E G K V S V S V  
 W L V M L L S L L K R G K L V L V \*  
 G W \* C Y \* V Y \* R G E S \* C \* C E  
 2385 2394 2403 2412 2421 2430  
 AAA AAG TCT GAT AAA ATG GTG TTA GAT AGT AAG TTG TTG TTA GAG GTT TGT GTT  
 K K S D K M V L D S K L L L E V C V  
 K S L I K W C \* I V S C C \* R F V L  
 K V \* \* N G V R \* \* V V V R G L C \*

GGGW = GGGAT  
 (Gly)  
 ACY = ACCCT  
 (Thy)  
 XAG  
 TCR = TCA/g  
 (Ser)

490  
 1980  
 490  
 1490  
 3  
 497A

2439 2448 2457 2466 2475 2484  
AGC GAC ACG GGA ATA GGT ATA GAG AAA GAC AAA TTG GGA TTG ATT TTC GAT ACC  
-----  
S D T G I G I E K D K L G L I F D T  
A T R E \* V \* R K T N W D \* F S I P  
R H G N R Y R E R Q I G I D F R Y L

2493 2502 2511 2520 2529 2538  
TTC TGT CAA GCT GAT GGT TCT ACT ACA AGA AAG TTT GGT GGT ACA GGT TTA GGG  
-----  
F C Q A D G S T T R K F G G T G L G  
S V K L M V L L Q E S L V V Q V \* G  
L S S \* W F Y Y K K V W W Y R F R V

2547 2556 2565 2574 2583 2592  
TTG TCA ATT TCC AAA CAG TTG ATA CAT TTA ATG GGT GGA GAG ATA TGG GTT ACT  
-----  
L S I S K Q L I H L M G G E I W V T  
C Q F P N S \* Y I \* W V E R Y G L L  
V N F Q T V D T F N G W R D M G Y F

2601 2610 2619 2628 2637 2646  
TCG GAG TAT GGA TCC GGR TCA AAC TTT TAT TTT ACG GTG TGC GTG TCG CCA TCT  
-----  
S E Y G S G S N F Y F T V C V S P S  
R S M D P X Q T F I L R C A C R H L  
G V W I R X K L L F Y G V R V A I \*

2655 2664 2673 2682 2691 2700  
AAT ATT AGA TAT ACT CGA CAA ACC GAA CAA TTG TTA CCA TTT AGT TCC CAT TAT  
-----  
N I R Y T R Q T E Q L L P F S S H Y  
I L D I L D K P N N C Y H L V P I M  
Y \* I Y S T N R T I V T I \* F P L C

2709 2718 2727 2736 2745 2754  
GTG TTA TTT GTA TCG ACT GAG CAT ACT CAA GAA GAA CTT GAT GTG TTG AGA GAT  
-----  
V L F V S T E H T Q E E L D V L R D  
C Y L Y R L S I L K K N L M C \* E M  
V I C I D \* A Y S R R T \* C V E R W

2763 2772 2781 2790 2799 2808  
GGA ATT ATA GAA CTT GGA TTG ATA CCT ATA ATA GTG AGA AAT ATT GAA GAT GCA  
-----  
G I I E L G L I P I I V R N I E D A  
E L \* N L D \* Y L \* \* \* E I L K M Q  
N Y R T W I D T Y N S E K Y \* R C N

2817 2826 2835 2844 2853 2862  
ACA TTG ACT GAG CCG GTG AAA TAT GAT ATA ATT ATG ATT GAT TCG ATA GAG ATT  
-----  
T L T E P V K Y D I I M I D S I E I  
H \* L S R \* N M I \* L \* L I R \* R L  
I D \* A G E I \* Y N Y D \* F D R D C

2871 2880 2889 2898 2907 2916  
GCC AAA AAG TTG AGG TTG TTA TCG GAG GTT AAA TAT ATT CCG TTG GTT TTG GTC  
-----  
A K K L R L L S E V K Y I P L V L V  
P K S \* G C Y R R L N I F R W F W S  
Q K V E V V I G G \* I Y S V G F G P

2925 2934 2943 2952 2961 2970  
CAT CAT TCT ATT CCA CAG TTG AAT ATG AGA GTA TGT ATT GAT TTG GGG ATA TCT  
-----  
H H S I P Q L N M R V C I D L G I S  
I I L F H S \* I \* E Y V L I W G Y L  
S F Y S T V E Y E S M Y \* F G D I F

2979 2988 2997 3006 3015 3024  
TCC TAT GCA AAT ACG CCA TGT TCG ATC ACG GAC TTG GCC AGT GCG ATT ATA CCA  
-----  
S Y A N T P C S I T D L A S A I I P  
P M Q I R H V R S R T W P V R L Y Q  
L C K Y A M F D H G L G Q C D Y T S

3033 3042 3051 3060 3069 3078  
GCG TTG GAG TCG AGA TCT ATA TCA CAG AAC TCA GAC GAG TCG GTG AGG TAC AAA  
-----  
A L E S R S I S Q N S D E S V R Y K  
R W S R D L Y H R T Q T S R \* G T K  
V G V E I Y I T E L R R V G E V Q N

3087 3096 3105 3114 3123 3132  
ATA TTA CTA GCA GAG GAC AAC CTC GTC AAT CAG AAA CTT GCA GTT AGG ATA TTA  
-----  
I L L A E D N L V N Q K L A V R I L  
Y Y \* Q R T T S S I R N L Q L G Y \*  
I T S R G Q P R Q S E T C S \* D I R

3141 3150 3159 3168 3177 3186  
GAA AAG CAA GGG CAT CTG GTG GAA GTA GTT GAG AAC GGA CTC GAG GCG TAC GAA  
-----  
E K Q G H L V E V V E N G L E A Y E  
K S K G I W W K \* L R T D S R R T K  
K A R A S G G S S \* E R T R G V R S

3195 3204 3213 3222 3231 3240  
GCG ATT AAG AGG AAT AAA TAT GAT GTG GTG TTG ATG GAT GTG CAA ATG CCT GTA  
-----  
A I K R N K Y D V V L M D V Q M P V  
R L R G I N M M W C \* W M C K C L \*  
D \* E E \* I \* C G V D G C A N A C N

3249 3258 3267 3276 3285 3294  
ATG GGT GGG TTT GAA GCT ACG GAG AAG ATT CGA CAA TGG GAG AAA AAG TCT AAC  
-----  
M G G F E A T E K I R Q W E K K S N  
W V G L K L R R R F D N G R K S L T  
G W V \* S Y G E D S T M G E K V \* P

3303 3312 3321 3330 3339 3348  
CCA ATT GAC TCG TTG ACG TTT AGG ACT CCA ATT ATT GCC CTC ACT GCA CAC GCC  
-----  
P I D S L T F R T P I I A L T A H A  
Q L T R \* R L G L Q L L P S L H T P  
N \* L V D V \* D S N Y C P H C T R H

3357 3366 3375 3384 3393 3402  
ATG TTA GGT GAT AGA GAA AAG TCA TTG GCC AAG GGG ATG GAC GAT TAT GTG AGT  
-----  
M L G D R E K S L A K G M D D Y V S  
C \* V I E K S H W P R G W T I M \* V  
V R \* \* R K V I G Q G D G R L C E \*

3411 3420 3429 3438 3447 3456  
AAG CCA TTG AAG CCG AAA TTG TTA ATG CAG ACG ATA AAC AAG TGT ATT CAT AAT  
-----  
K P L K P K L L M Q T I N K C I H N  
S H \* S R N C \* C R R \* T S V F I I  
A I E A E I V N A D D K Q V Y S \* Y

3465 3474 3483 3492 3501 3510  
ATT AAC CAG TTG AAA GAA TTG TCG AGA AAT AGT AGG GGT AGC GAT TTT GCA AAG  
-----  
I N Q L K E L S R N S R G S D F A K  
L T S \* K N C R E I V G V A I L Q R  
\* P V E R I V E K \* \* G \* R F C K E

3519 3528 3537 3546 3555 3564  
AAG ATG ACC CGA AAC ACA CCC GGA AGC ACG ACC CGT CAG GGG AGT GAT GAG GGG  
-----  
K M T R N T P G S T T R Q G S D E G  
R \* P E T H P E A R P V R G V M R G  
D D P K H T R K H D P S G E \* \* G E

3573 3582 3591 3600 3609 3618  
AGT GTA AAG GAC ATG ATT GGG GAC ACT CCC CGT CAA GGG AGT GTG GAG GGA GGG  
-----  
S V K D M I G D T P R Q G S V E G G  
V \* R T \* L G T L P V K G V W R E G  
C K G H D W G H S P S R E C G G R G

3627 3636 3645 3654 3663 3672  
GGT ACA AGT AGT AGA CCA GTA CAG AGA AGG TCT GCC AGG GAG GGG TCG ATC ACT  
-----  
G T S S R P V Q R R S A R E G S I T  
V Q V V D Q Y R E G L P G R G R S L  
Y K \* \* T S T E K V C Q G G V D H Y

3681 3690 3699 3708 3717 3726  
ACA ATT AGT GAA CAA ATC GAC CGT TAG CTA ACG ACT CAA GCT GAC GCT TGA GTC  
-----  
T I S E Q I D R \* L T T Q A D A \* V  
Q L V N K S T V S \* R L K L T L E S  
N \* \* T N R P L A N D S S \* R L S Q

3735 3744 3753 3762 3771 3780  
AAA GCT ACA AAT ATT TAG CCA ATT GTT TAC TTA GAT AAA TAA AAT ACA AGT AAA  
-----  
K A T N I \* P I V Y L D K \* N T S K  
K L Q I F S Q L F T \* I N K I Q V N  
S Y K Y L A N C L L R \* I K Y K \* T

3789 3798 3807 3816 3825 3834  
CCA TTG TTG TGT TTA GAT CAA TAA TTG AAA AAT AAA CAA GAT TAC TAA AAA TAT  
-----  
P L L C L D Q \* L K N K Q D Y \* K Y  
H C C V \* I N N \* K I N K I T K N I  
I V V F R S I I E K \* T R L L K I S

3843 3852 3861 3870 3879 3888  
CAA GCC AAA TTG TTG TTG CAG GAG CTG GGT TTT TGG GKG GGG TTA ACT TTT TTA  
-----  
Q A K L L L Q E L G F W X G L T F L  
K P N C C C R S W V F G X G \* L F Y  
S Q I V V A G A G F L G G V N F F T

540  
486

134

1070AA

1250  
1070  
180x3  
5403696  
487

3009

136

1357

1062

3897 3906 3915 3924 3933 3942  
CCA AAA AAT GGA TAA AAA AGG GGA TGT GAT CCA AGT AGT AAC TTT AGT GAC TGT  
P K N G \* K R G C D P S S N F S D C  
Q K M D K K G D V I Q V V T L V T V  
K K W I K K G M \* S K \* \* L \* \* L F

3951 3960 3969 3978 3987 3996  
TTA GGT TAC TTG AGC TAT CCA ATT AGA ATN TCM CCC CCC GCA GTA AGT TTG GTC  
L G Y L S Y P I R X S P P A V S L V  
\* V T \* A I Q L E X X P P Q \* V W S  
R L L E L S N \* N X X P R S K F G L

4005 4014 4023 4032 4041 4050  
TTA TTG TTT ACG GAA AAA TAA GAA ACC CTA GCC CTG GAC TAG CCC CTA CCT AGT  
L L F T E K \* E T L A L D \* P L P S  
Y C L R K N K K P \* P W T S P Y L V  
I V Y G K I R N P S P G L A P T \* F

4059 4068 4077 4086 4095 4104  
TTT GAA GTG AAA TTT TTT TTT TTT TAG TTG ACT CCC CCC CCC TAG ACC AAT  
F E V K F F F F F \* L T P P P \* T N  
L K \* N F F F F F S \* L P P P R P I  
\* S E I F F F F L V D S P P L D Q S

4113 4122 4131 4140 4149 4158  
CGA AAG CCG TGG TAT TAT GCC GGG CAT AGA AAA AAA TCT TTC TTT TTT CTT TTT  
R K P W Y Y A G H R K K S F F F L F  
E S R G I M P G I E K N L S F F F F  
K A V V L C R A \* K K I F L F S F F

4167 4176 4185 4194 4203 4212  
TTT GGT GTG GGC CCC CAG TTT ATG CAA CAT CAC TTA ACC CCC CTC AGC CAA AAA  
F G V G P Q F M Q H H L T P L S Q K  
L V W A P S L C N I T \* P P S A K K  
W C G P P V Y A T S L N P P Q P K K

4221 4230 4239 4248 4257 4266  
AAA CCA TTT AAT TAT CCT TCC CCC CCC CCG CGG ATG AAG AAA ACC NAA CCA AAA  
K P F N Y P S P P P R M K K T X P K  
N H L I I L P P P P R G \* R K P N Q K  
T I \* L S F P P P A D E E N X T K K

4275 4284 4293 4302 4311 4320  
AAA ANT TIN TTT TTT TTT TCC CCN CCN CCT NTC TTT TCC AAC AAC CAC ATT TNC  
K X X F F F S P P P X F S N N H I X  
K X X F F F P X X L S F P T T T F X  
X F X F F F P X X X L F Q Q P H X X

4329 4338 4347 4356 4365 4374  
NTN TCG GAT CCC CCC ACC TGA TTT CAA ACT NAA AAN ACN GGC GGG CCT TTT TNT  
X S D P P T \* F Q T X X T G G P F X  
X R I P P P D F K L K X X A G L F X  
X G S P H L I S N X K X X R A F X F